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FATE AND EFFECTS OF PENTACHLOROPHENOL IN HARD- AND SOFT-WATER MICROCOSMS

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ABSTRACT

The influence of hard water and soft water on the fate and effects of pentachlorophenol (PCP) was investigated in small microcosms. Minor differences in the fate of PCP and minor changes in microcosm structure were noted between the hard- and soft-water systems. Definite differences between hard-and soft-water systems were detected in dissolved oxygen production and dissolved silica concentration at an exposure concentration of 4 mg l^{-1} PCP.

INTRODUCTION

Water quality variations in natural waters (site-specitic factors) are known to influence the fate and effects of inorganic pollutants such as heavy metals and ammonia (1). Relative to organics, however, the influence of the physical and chemical properties of aquatic environments on fate and effects processes and the functions of biological communities has not been evaluated thoroughly. Consequently, predictions of the fate and impact of organic pollutants in freshwater ecosystems include an unknown level of uncertainty. Before environmental decision-makers can have confidence in site-specific criteria and predictive fate and effects models, researchers must determine how much influence various physical and chemical conditions have on pollutant behavior.

The objective of this study was to determine the influence of hardness and alkalinity on the biotic communities and on the fate and effects of pentachlorophenol (PCP) in small laboratory ecosystems. Pentachlorophenol was chosen as the test compound because of its widespread occurrence in varied ecosystems (2) and its potential to undergo biodegradation (3) and photodegradation (4). The study was performed with mixed biota communities in both continuous-flow and static aquaria.

MATERIALS AND METHODS

Continuous-flow and static experiments were carried out in seamless glass aquaria (20 cm X 30 cm X 18 cm) under Vitalite® fluorescent bulbs at an intensity of 550 lux with a 12-hr photoperiod. Water temperature varied from 20°C at lights-on to 24.5°C at lights-off. The apparatus and the flow and mixing conditions have been described by Brockway et al. (5). PCP stock solutions were made up using pentachlorophenol-Ring-UL-^{14}C from Pathfinder Laboratories, Inc., and unlabeled pentachlorophenol from Sigma Chemical Co., in 0.1N NaOH to form a sodium pentachlorophenate stock. (Anytime PCP is mentioned in this paper it refers to the ionic form.)

Initially, each microcosm received a 0.5-cm layer of sediment, 7 l of water, and an inoculum of net plankton plus aufwuchs from two mesotrophic, soft-water, 2-ha ponds. Subsequently, each microcosm received a weekly inoculum of net plankton from the two ponds during the development phase of the experiment (80 days). Nutrient medium for the soft-water microcosms was modified Freeman's standard reference water (6). Hard-water microcosms received this medium but with a 10-fold increase in $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and $NaHCO_3$ stocks. Twenty-four microcosms were organized into 8 sets of 3--half with hard medium, half with soft medium. Each triplicate had a separate nutrient feed system. The hardness and alkalinity were 18 and 14 mg l^{-1}, respectively, for the soft-water medium and 180 and 140 mg l^{-1} for the hard-water medium.

After 80 days, 18 of the microcosms were dosed with PCP. Treatments were 3 hard and 3 soft flow tanks at 0 mg l^{-1} (controls), 3 hard and 3 soft flow tanks at 0.2 mg l^{-1}, 3 hard and 3 soft flow tanks at 1.0 mg l^{-1}, and 3 hard and 3 soft static tanks at 1.0 mg l^{-1}. An initial spike of PCP brought the 7-l volume in appropriate aquaria to the desired initial test compound concentration. After the initial spike, the hard and soft static tanks received no additional input of any kind. Four additional static control tanks were established at this time--hard-water light, hard-water dark, and soft-water light and dark. Only medium amended to 1.0 mg l^{-1} PCP was added to these tanks and the only organisms present were those that entered from the room air.

In the flowing 1.0 mg l^{-1} treatment, the concentration of PCP was changed to 1.5 mg l^{-1} on day 23 of the exposure phase, to 2.0 mg l^{-1} on day 30, to 3.0 mg l^{-1} on day 35, and to 4.0 mg l^{-1} on day 37. Whenever the NaOH solutions of PCP were added to the systems, an equivalent amount of HCl was added to neutralize the additions. The experiment was terminated on day 40 of the exposure phase.

Additional static tests were carried out using 250-ml beakers containing 200 ml of the hard-water medium at 1.0 mg l^{-1} PCP. The beakers were inoculated with 0.5-g aliquots (wet weight) of mixed biota and 40 g of sediments from the same ponds. Temperature and lighting were the same as with the flow studies.

Nine parameters were monitored throughout the experiment. Dissolved oxygen (DO) and hydrogen ion activity (pH) were measured before and after the 12-hr light period using an Orbisphere DO meter and an Orion 231 digital pH meter. Samples to be analyzed for soluble reactive phosphorus and dissolved nitrate, nitrite, ammonia, and silica were taken on the same day each week and at the same time (1 hr past lights-on) each day and were analyzed using a Technicon Auto

Analyzer II (7). Hardness and alkalinity were determined weekly using standard methods (8), that is, EDTA Titrametric Method 314B and Alkalinity Method 403.

Samples for PCP analysis were acidified and extracted in methylene chloride using a Polytron® apparatus. Samples were evaporated to near dryness and redissolved in 25% water-methanol. PCP was analyzed by high-pressure liquid chromatography using a 25% water-methanol mobile phase on a Whatman Portasil ODS-2 column and a variable wavelength UV-VIS detector at 254 nm. ^{14}C-PCP and products in water were counted in 1-ml samples in Aquasol II. Biota and sediment samples were oxidized in a Harvey oxidizer and $^{14}CO_2$ was collected in Harvey cocktail. All samples were counted in a liquid scintillation spectrometer.

Relative abundance of algal genera was estimated by direct counts with an inverted microscope. Samples were preserved in Lugol's solution and refrigerated.

RESULTS AND DISCUSSION

Community Development and Functions before PCP Exposure

Observations and counts revealed little difference in autotrophic components between hard- and soft-water microcosms at the end of the pre-exposure phase of development. The most numerous algal genera in all systems were Chroococcus, Oscillatoria, Oedogonium, Synedra, Fragilaria, and Scenedesmus. The only observed differences between hard and soft systems were that Ankistrodesmus was numerous only in soft-water systems and Navicula, Nitzschia, and Anabaena were numerous in the hard-water systems. The latter is consistent with an observation by Prescott (9) that drainage from hard-water areas generally has a flora richer in diatoms and blue-greens. No counts were made of heterotrophic organisms, but, observations indicated several groups were present in all tanks throughout the pre-exposure period.

Functionally, also, the systems showed little difference between treatments. Oxygen production increased gradually throughout the pre-exposure period to a change of 8 mg l^{-1} between morning and evening with no significant difference between hard and soft systems. Morning DO concentrations decreased from 8 mg l^{-1} for week 1 to 6.5 mg l^{-1} for week 11 and evening DO increased from 11 to 14.5 mg l^{-1} by week 11. This pattern of development was similar to that observed in several prior experiments of this type (e.g. 5) and served as justification to begin perturbation during week 11.

Fluctuations in pH were much greater in soft-water systems than in hard due to the difference in buffering capacity. The diurnal change in pH was from 7.0 in the morning to 9.5 in the evening in the soft tanks and from 8.0 to 9.0 in the hard tanks. Analysis of nutrients indicated no significant differences in concentrations of nitrate, nitrite, ammonia, and soluble reactive phosphorus (SRP) in the waters of hard and soft tanks. Concentrations after week 4 were generally below 0.010 mg l^{-1} for nitrate, below 0.002 mg l^{-1} for nitrite, below 0.020 mg l^{-1} for ammonia, and below 0.010 mg l^{-1} for SRP. These findings were similar to values from previous studies in similar systems.

One nutrient, however, did show interesting, although not significant, differences between hard and soft tanks. Dissolved silica (SiO_2) concentrations in individual tanks within treatments were variable, but the mean of the hard-water tanks was 1.8 mg l^{-1} (CV=75%) and the mean

of the soft-water tanks was 6.8 mg l^{-1} (CV=59%) at the end of the pre-exposure phase. The input concentration was 13.2 mg l^{-1} and the difference between treatments, although not statistically significant, probably reflects greater diatom activity in the hard-water systems, because no simple chemical exchanges or reactions have been found that could explain the differences.

In general, these results again reinforce the concept of equifinality (10). To test the concept of water quality influencing the functioning of systems, however, additional work in laboratory systems using inocula from natural areas with physical-chemical conditions comparable to the treatment parameters needs to be done.

Fate of PCP

The fate of PCP in this study was determined by comparing the amounts of parent PCP and PCP-degradation products remaining in the aqueous phase of the systems tested. As expected (11), photolysis was the main degradation process (Table 1) for at least 15 days and probably for 22 days in this study. This was indicated by comparing the water-only control tanks (which were static) with the 1 mg l^{-1} static tanks for days 4, 8, and 15 where the data were complete. Because essentially no biota (thus no sorption) were present in the water-only tanks, a calculation was made to compare the data. In the dosed static tanks, both PCP and its products were removed from the water by processes that, in total, will be referred to as sorption. Therefore, only the fractions in the water (not the loss value) should be compared to the control tanks.

If the PCP value is divided by the PCP plus products total for the dosed tanks and multiplied by 100, the result should be comparable to the PCP value of the water-only tanks. The calculated values were 79 and 77, 73 and 70, and 42 and 43 for the soft and hard tanks for days 4, 8, and 15, respectively. When these are compared to the PCP values for the water-only tanks (91 and 74, 67 and 54, 47 and 28), it can be seen that the comparable values are similar, and there was no indication that processes other than photolysis contributed much to the degradation of PCP.

The water-only tanks were not sterile and growth of biota did occur in them after 2 to 3 weeks. This biomass was not quantified but it did not appear to be enough to significantly contribute to any processes. It must be noted again that the water-only controls were single tanks: 1 hard and 1 soft. All other values in Table 1 are means of triplicate tanks and the coefficients of variation (CV) were low. The CV for the ^{14}C-PCP plus products measurements were all below 11% except for soft-water 1 mg l^{-1} flow (15.5%) and static (18.9%) tanks on day 22 and all but 7 of the CV were below 8%. The CV value for the PCP measurements were all below 16% except for four times when the concentrations were getting low in the 0.2 mg l^{-1} and static systems.

The photolytic half-life of PCP of 10-15 days in this study was much slower than the 5 hr measured in flasks by Wong and Crosby (4) and the half-life of 4.2 hr in vials placed 3.8 cm under water measured by Pignatello et al. (11) both in full sunlight. These differences are not surprising because the light energy to our microcosms at the wavelength most photoactive for PCP (320 nm) was only 10^{-2} $\mu W\ cm^{-2}\ nm^{-1}$ whereas peak sunlight at 320 nm is about 4 $\mu W\ cm^{-2}\ nm^{-1}$ or 400 times greater.

Table 1. Pentachlorophenol (PCP) and products remaining in water as a percentage of initial dosing concentration along with total ^{14}C recovered (percent) from the complete systems. For all treatments n=3, except water-only where n=1.

		Low Hardness Systems				High Hardness Systems			
Day	Fraction	Water Only	1 mg l^{-1} Flow	1 mg l^{-1} Static	0.2 mg l^{-1} Flow	Water Only	1 mg l^{-1} Flow	1 mg l^{-1} Static	Flow
2	PCP	93	78	74	72	80	89	79	73
	Products[a]	7	8	4	0	20	b	b	b
	Loss[a]	0	14	22	28	0	20	25	28
4	PCP	91	79	55	63	74	70	50	52
	Products	9	5	15	7	26	5	15	16
	Loss	0	16	30	30	0	25	35	32
8	PCP	67	65	38	42	54	55	32	45
	Products	33	14	14	16	46	16	14	17
	Loss	0	21	48	42	0	29	54	38
15	PCP	47	62	14	33	28	54	12	35
	Products	46	15	19	13	59	13	16	21
	Loss	7	23	67	46	13	33	72	44
22	PCP	22	65	--[c]	34	20	44	--[c]	33
	Products	51	10	--[c]	15	45	21	--[c]	20
	Loss	27	25	76	51	35	35	84	47
End	Total ^{14}C recovered from systems -- %		99	102	99		96	90	99

a Values for products and loss are calculated; loss = 100 - the total ^{14}C material expressed as PCP; products = 100 - (loss + PCP)

b The ^{14}C measurements or loss fractions for these times are consistent with the values for the times thereafter. The PCP values are not, they are high. Therefore, the products values could not be calculated.

c PCP values below detectability, so products could not be calculated.

Biodegradation did not appear to be a significant process in these systems at least for the first 22 days (i.e., constant input phase) as evidenced above in the photolysis discussion. During the rest of the study, when the PCP concentration was increased in steps, the half-life of PCP after each introduction, even with the constant input at the new concentration, was similar to the half-life during the first phase. In other words, no large increase occurred in the degradation rate due to increased biodegradation after 3 weeks exposure, contrary to the findings reported for outdoor channels by Pignatello et al. (11).

The ultimate disposition of the PCP and degradation products in the experimental system in this study was interesting. The mass balance calculations indicated that essentially no ^{14}C was lost from the systems, whereas ^{14}C was lost from water in the water-only controls after 15 and 22 days. Because no sediments and very little biota were in these tanks, either ^{14}C products escaped or the few biota present were very efficient sorbers (the latter a highly unlikely occurrence). Of the 18 tanks that were dosed, the recoveries for 13 ranged from 96 to 103%; recoveries for the other 5 (all static tanks) were 74, 87, 94, 108 and 112%. One explanation for the greater variation in the static tanks is simply that there was much less total activity added to the tanks as a one-time dose (1750 nCi) as compared to the totals added to the flowing 0.2 mg l^{-1} tanks (8,230 nCi) and 1 mg l^{-1} tanks (27,000 nCi).

The near 100% recovery of ^{14}C indicates that essentially no $^{14}CO_2$ was lost from the systems even though PCP is significantly mineralized to CO_2 both photolytically (4) and microbially (11). The most probable explanation of the results in our systems is that the heavily autotrophic biomass was utilizing the $^{14}CO_2$ before it was lost from the system.

Results slightly different from these were obtained in a beaker study done to determine the relative contributions of biota and sediments to the loss of PCP and photolysis products from hard-water systems in the light compared to loss of PCP only in the dark. All the treatments were in triplicate and the CV were 15% or less for all water, sediment, and biota samples. In these beakers, whether they contained biota, sediments, neither, or both, from 5 to 8% of the ^{14}C was unaccounted for at the end of the 7-day study (Table 2). The only exception was the water-only treatment in the dark where all the ^{14}C was still in the water. The loss of ^{14}C from the systems in the light may be explained by the fact that only 7% as much biota per volume of water was in the beaker as was in the larger microcosms, thus less uptake of the $^{14}CO_2$ from mineralized PCP occurred. The loss of ^{14}C from the beakers in the dark is more difficult to understand, however, because obviously no photolysis occured and the organisms had no known previous exposure to PCP that could have induced degradation. One parameter of potential importance to PCP fate, pH, did fluctuate some but probably was not a factor here because it remained in the range where PCP stays in the ionic form. The systems stayed between a pH of 7.1 and 7.7 except for all controls, which started at 7.5 but stayed at 8.3 after day 2, and the biota in the light treatment, which fluctuated up to pH 10.5 by day 7. None of these pH differences were correlated with differences in ^{14}C-levels.

Table 2. Fate of PCP plus products in static 250-ml beakers in light or dark. Results are expressed as percentage loss from water of radioactivity present initially in the water phase and percentage gain of that initial activity by the other phases. Three beakers were sacrificed for each treatment at each time. The unaccounted for value is the difference between the water value and the sorption value in percent.

Treatment	Fraction	Day 1	Day 2	Day 4	Day 7	Unaccounted for on Day 7
Water Only, Dark	H_2O (loss)	0	0	0	0	0
Water Only, Light	H_2O (loss)	0	0	0	6	6
Sediment, Dark	H_2O (loss)	22	33	42	52	5
	Sediment (gain)	23	32	45	47	
Sediment, Light	H_2O (loss)	25	37	51	67	8
	Sediment (gain)	25	33	46	59	
Biota, Dark	H_2O (loss)	8	8	7	11	7
	Biota (gain)	4	4	4	4	
Biota, Light	H_2O (loss)	6	9	13	18	7
	Biota (gain)	4	6	8	11	
Sediment + Biota, Light	H_2O (loss)	30	39	50	62	8
	Sediment (gain)	25	38	36	43	
	Biota (gain)	6	7	8	11	

The beaker study furnished interesting information relative to the objectives for running it. First the relative differences between water and other components in light and dark for both biota and sediments was about the same, which indicates PCP-products were sorbed at least as fast as parent PCP. Second, total sorption in the biota plus sediments was about the same as sorption in the sediments alone, and the biota fraction of the combination treatment was similar to the biota-only treatment; thus, the difference was in the sediment fraction of the combination. This finding could be explained by the fact that the biota formed a mat over the sediments that created a barrier to direct contact with PCP in the overlying water column.

The portion of the PCP input that was sorbed by the biota and sediment in the complex microcosms was significant. In the 6 static tanks, the total PCP sorbed ranged from 62 to 91% of the input and was approximately equal in each fraction. In the 6 low and 6 high flowing tanks, the PCP ranges were 40 to 46% and 30 to 54%, respectively, with the biota having from 1 to 3 times and 2 to 4 times as much as the sediments, respectively. These removal values are much higher than the <15% values obtained in an outdoor channels study (11) and also higher than found by Boyle et al. (12) in 50-l aquaria, but the differences were probably due to the large amount of biomass (up to 300 g wet weight of biota in a 7-l tank) and the 8-day retention time of our systems.

The uptake by biota was quite uniform and bioconcentration factors (BCF) were calculated using the wet weight of the biota relative to the highest concentration to which they had been

exposed because the concentrations varied depending on the treatment. The mean BCF values were 73, 53, and 40 for the low-PCP flowing, high-PCP flowing and high-PCP static soft water systems, respectively, and 46, 42, and 18 for the respective treatments in hard-water systems. These values are in contrast to a reported BCF of 1250 for an alga Chlorella fusca in culture flasks (13).

The main objective of this study was to determine the differences in response of systems to high- or low-hardness and alkalinity water but the results make it difficult to draw any definite conclusions regarding these parameters and the fate of PCP. It appears that PCP was degraded faster for the first 15 days in hard water in the water-only controls (Table 1) but by day 22 PCP values in both tanks were similar. In a similar previous microcosm study, run for 8 days, similar differences were observed in hard- and soft-water results, but in both studies there were no replicate water-only controls. In the 1-mg l^{-1} flow tanks, PCP disappeared from water faster in hard water systems than in soft, but it is difficult to tell whether it was from degradation or sorption. In the preliminary 8-day study, PCP in water decreased significantly faster ($P=0.05$) in the soft-water systems than in the hard, so the results are conflicting.

Essentially no differences were found in the results from the hard and soft 1-mg l^{-1} static systems nor in the hard and soft 0.2-mg l^{-1} systems, which would be closer in concentration to a potential PCP occurrence in the environment. Thus, the overall conclusion must be that there were no differences in the fate of PCP under high or low alkalinity and hardness in this study.

Effects of PCP

The influence of hard- and soft-water on the effects of PCP in this study was evaluated in terms of the biota, nutrients, and functions in the test systems.

Structure of the systems

Of the six dominant genera of algae present in all systems during the pretreatment phase of the study, none changed in abundance relative to the controls during the exposure phase. Major changes in abundance did occur in three genera of algae that were abundant but not dominant during the pretreatment phase. In the hard-water systems, the diatom Nitzschia decreased markedly in both the low- and high-PCP treatment tanks, whereas the blue-green Anabaena did not change in the low but almost disappeared in the high-PCP tanks. Neither of those genera was present in the soft-water systems. In the soft-water systems only Ankistrodesmus, which was not present in the hard-water systems, decreased markedly, but only in the high-PCP systems.

Only gross observations of organisms present at termination of the experiment were available for the heterotrophic populations, but the results were quite clear. Both the control and the 0.2-mg l^{-1} PCP systems with hard and soft waters had similar expected complements of zooplankters and burrowing benthic organisms, whereas none of either group was observed in either hard- or soft-water systems exposed to the high levels of PCP (4 mg l^{-1}).

The total biomass measured as ash-free dry weight at termination of the study did not indicate any difference between any treatments. For controls, low-PCP flow treatments, and high-PCP flow treatmencs, the mean values for the soft-water tanks were 4.35, 4.11, and 3.88,

respectively, and 4.53, 4.21 and 3.48 g for the hard-water tanks. The static tanks had 3.31 g for the hard and 2.59 g for the soft-water treatments, but no input of nutrients occurred during the static phase and the systems were probably nutrient limited.

In summary, no statistically significant difference was found in the amount of biomass generated in any of the flow systems. At the high concentrations of PCP in both hard and soft systems, however, a distinct impact was evident on the heterotrophic community and a modest alteration occurred in the autotrophic structure.

Nutrients in the system

Examination of the concentrations of the major dissolved nutrients (nitrate, nitrite, ammonia, and SRP) revealed no meaningful information about the impact of PCP on the systems. Some isolated instances of significant differences between concentrations for different treatments were apparent, but the absolute values (e.g., 0.005 and 0.009 µg l^{-1} NO_3-N) were so low and the populations were still utilizing so much that it would be presumptuous to attribute any meaning to the differences.

The concentrations of dissolved silica also did not change from pre-exposure phase levels in either the control or low-PCP exposure systems (Table 3). In the high-PCP systems, however, changes in silica occurred that appeared to depend on PCP concentration and water type. During the 23 days when the exposure level was 1 mg l^{-1}, the silica in the hard-water systems increased to 11.3 mg l^{-1} (from about 1.8 mg l^{-1} during pre-exposure), but stayed relatively constant (6 to 7 mg l^{-1}) in the soft-water systems. This difference suggests that a system with more diatoms (hard water) could be more sensitive to PCP than one with fewer (soft water).

Table 3. Concentrations of dissolved silica in flowing hard- and soft-water microcosms from beginning to end of PCP-exposure. All values are expressed as mg $l^{-1}SiO_2$ with n=3 and the CV in parentheses.

	Day of Exposure Phase						
Treatment	0	2	9	16	23	30	37
Hard							
Controls	2.1 (123)	1.0 (46)	1.1 (38)	3.3 (110)	0.8 (30)	0.8 (20)	0.6 (18)
Low PCP	0.7 (31)	0.7 (61)	0.7 (69)	0.8 (76)	0.8 (67)	0.3 (43)	0.2 (137)
High PCP	2.3 (8.7)	4.9 (28)	8.6 (19)	10.2 (13)	11.3 (13)	14.5 (5.6)	17.2 (5.4)
Soft							
Controls	5.5 (68)	5.3 (65)	4.8 (86)	1.0 (39)	1.9 (142)	1.2 (131)	0.9 (109)
Low PCP	3.8 (84)	4.5 (104)	4.5 (131)	4.4 (151)	4.4 (163)	4.5 (160)	4.3 (163)
High PCP	6.2 (46)	6.0 (52)	5.5 (89)	6.8 (81)	7.2 (90)	11.4 (42)	14.7 (28)

When the PCP concentration was increased to 1.5 mg l^{-1}, the silica concentrations increased to 14.5 and 11.4 mg l^{-1} for the hard- and soft-water systems, respectively. When the PCP was raised to 2 and then 3 mg l^{-1} before the next silica sampling, the silica levels increased to 17.2 and 14.7 mg l^{-1} for hard- and soft-water systems, respectively. At these higher PCP levels, hard- and soft-water systems showed no difference in response. No nutrient samples were taken

after the short 4 mg l^{-1} PCP input.

These results suggest that silica concentration may be a parameter that could indicate stress on a system. Two considerations should be pointed out here, however. First, the concentrations of PCP that had an impact on these systems were higher than have been shown to be detrimental to other organisms, e.g., fathead minnows for which growth was inhibited at 73 µg l^{-1} (14). A study in a small pond found results similar to ours on heterotrophs and autotrophs at a concentration of 1 mg l^{-1} (15). Second, only a small portion of the metabolism of the systems may have been impacted (indicated by oxygen production, discussed in the next section) with the overall function of the systems not being influenced except at the highest PCP concentration tested.

Impact on functions

The two most reliable and easiest to measure parameters we have found for monitoring system functions are pH and DO, measured in the morning and the evening. In this study, when the systems were exposed to PCP, neither parameter changed much from the pre-exposure phase until the highest PCP exposure (4 mg l^{-1}) was begun.

The pH of both hard-water and soft-water systems remained approximately where they had been at the end of the pre-exposure phase until day 37 of the exposure phase (the middle of week 16) when the 4 mg l^{-1} exposure was begun. The diurnal changes in pH were still from about 7.0 in the morning to 9.5 in the evening in the soft tanks and from 8.0 to 9.0 in the hard tanks. The CV of almost all the sample means for both morning and evening readings were less than 4% with the few remaining values less than 10%. There was some indication of change in pH in the soft-water tanks after the 3 mg l^{-1} PCP treatment was begun, when the morning pH of all three tanks was 0.2 units lower and the evening pH of two of the three replicates was 0.9 units lower than two days earlier. On the following day the values had returned to the previous levels. No such pH decrease occurred in the hard-water systems.

When the 4 mg l^{-1} PCP exposure began, the morning and evening pH of all three replicates of the hard-water tanks decreased 0.3 units then returned to the normal levels over the next 4 days. In the soft-water systems, there were dramatic changes. On the morning after the PCP was increased, the pH of all three replicates was 0.5 units below the previous morning readings and the evening values were 1.3 units below the previous evening values. The next day the morning values had returned almost to their normal values, but by evening the replicates were starting to vary such that one had increased slightly, but the other two had decreased another 0.4 and 0.8 units, respectively. The pH of the latter tank was then 6.7 and it had been 6.3 that morning. These changes will be treated more fully in the discussion of dissolved oxygen. The pH of the systems over the next two days returned almost to the pretreatment levels. There is no question, however, that the soft-water systems were impacted more than the hard-water systems by 4 mg l^{-1} PCP.

The DO concentrations in all the systems undergoing all treatments followed patterns very similar to those of pH. The levels that existed at the end of the pretreatment phase were maintained with slight variation (with possible exception of one hard-water system) throughout

the PCP treatment phase until the last increase in PCP concentration occurred (Figures 1 and 2). The CV of all the flowing tanks were less than 23% until the last increase in PCP and for all control tanks less than 14%.

It appears that hard-water tank 17 (Fig. 1) may have been influenced by the last three PCP increases, but it is difficult to draw a definite conclusion. There is also an indication of some DO inhibition of the soft-water, high-PCP tanks (Fig. 2) after the 3 mg l^{-1} treatment, a finding that is supported by the pH data previously reported. Conclusive changes in DO occurred in both hard- and soft-water systems during the 4 mg l^{-1} PCP treatment, however. In the hard-water systems, the morning DO values did not change much, but the evening values decreased by from 2.4 to 4.8 mg l^{-1}. They then increased and by the fourth day were back to normal. In the soft-water systems, the DO values for the morning after the last increase of PCP were still typical, but, then the large changes occurred. In one tank (Number 5, Fig. 2), the DO barely increased during the day after the PCP increase, whereas in the other two, Numbers 7 and 12, the DO kept decreasing during the daylight hours for a net loss of oxygen. By the next morning the DO in all three tanks had further decreased, but, during the day net production again occurred and tank 5 was approaching normal again. During the next two days, one tank recovered and one remained inhibited.

The results were consistent with the hypothesis that PCP would inhibit autotrophs more than it would most heterotrophs, particularly microheterotrophs, so oxygen production was inhibited, but respiration continued. The concentration of PCP at which inhibition was detected (3 to 4 mg l^{-1}) in these systems was higher than seen in some previous studies, as has already been discussed. Whether this is due to insensitivity of these systems or to hypersensitivity of single-species tests is not known.

Similar results were obtained in a single-species algal assay performed early in this study (unpublished data) where 50% inhibition of production of Selenastrum occurred at 0.18 mg l^{-1} in soft-water medium and 0.84 mg l^{-1} in hard-water medium, both of which were lower concentrations than observed to be effective in this study. Greater sensitivity in soft-water also was observed in this study, possibly by sensitivity at a lower concentration, but, definitely by greater sensitivity at the same concentration in soft-water than in hard.

Another recent example of water quality influencing toxicity was presented by Launer and Bills (16) who concluded that, for rough-fish, control environmental factors needed to be taken into consideration for calculating dose and response. Their purpose of finding a concentration to purposely kill organisms was different than our study goal, but again it was a single species in laboratory tests.

Our study did not definitively answer the question of the relationship between the physical-chemical parameters of water and the fate and toxicity of organics in systems, and additional work needs to be done with different parameters and other toxicants in complex systems.

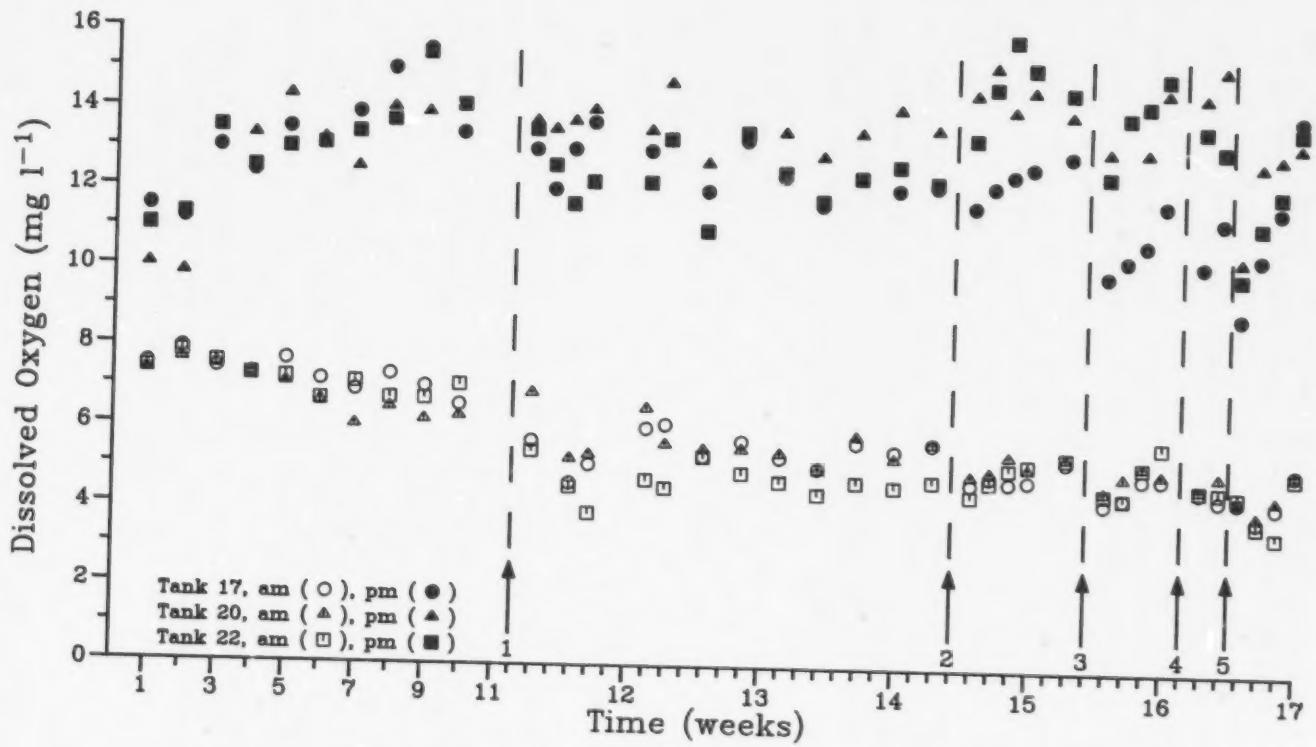

Figure 1. Morning and evening dissolved oxygen in hard-water, high-PCP treatment tanks Pre-exposure occurs in weeks 1-11, after which the time scale changes and the beginning of each week is labeled. Arrows indicate changes in exposure concentration.

Arrow 1 = 1.0 mg l^{-1} Arrow 2 = 1.5 mg l^{-1} Arrow 3 = 2.0 mg l^{-1}
Arrow 4 = 3.0 mg l^{-1} Arrow 5 = 4.0 mg l^{-1}

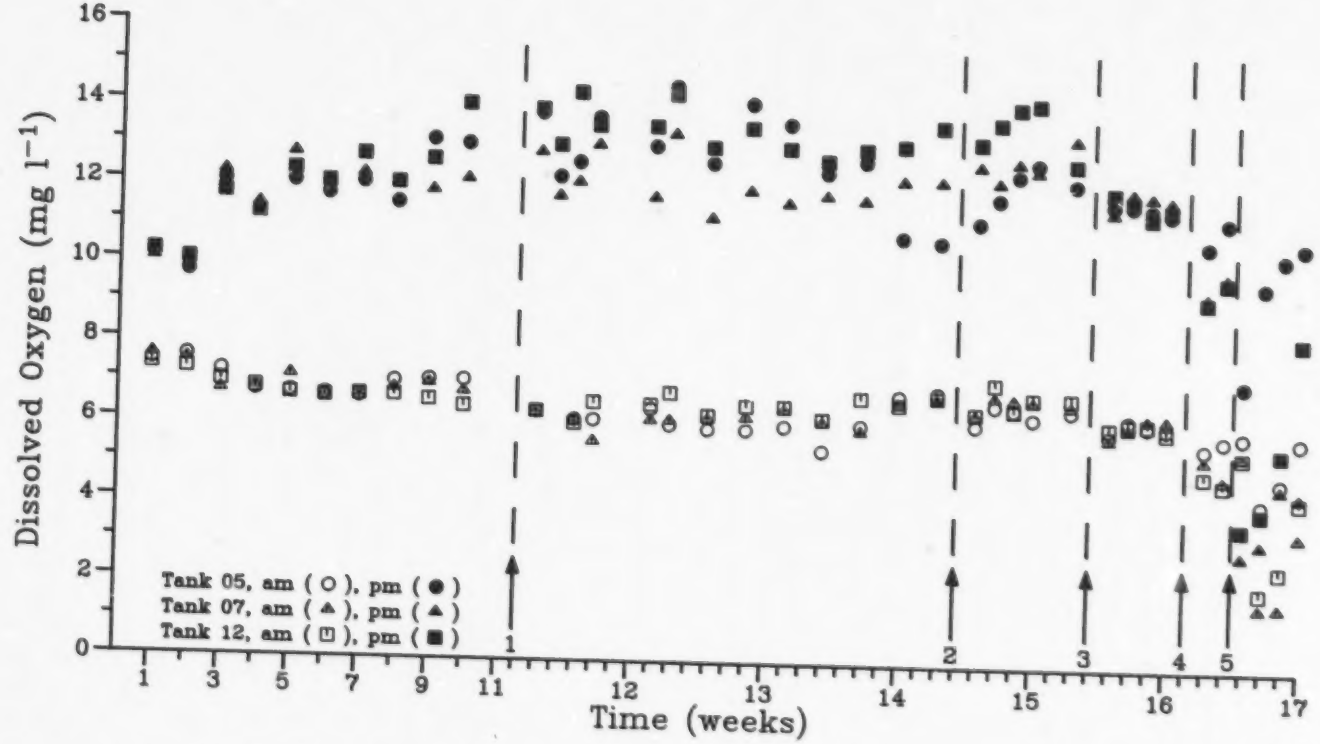

Figure 2. Morning and evening dissolved oxygen in soft-water, high-PCP treatment tanks Pre-exposure occurs in weeks 1-11, after which the time scale changes and the beginning of each week is labeled. Arrows indicate changes in exposure concentration.

Arrow 1 = 1.0 mg l^{-1} Arrow 2 = 1.5 mg l^{-1} Arrow 3 = 2.0 mg l^{-1}
Arrow 4 = 3.0 mg l^{-1} Arrow 5 = 4.0 mg l^{-1}

CONCLUSIONS

1. These laboratory ecosystems developed very similarly in both hard water and soft water with only minor differences in structure and function.
2. After exposure to PCP, system structure showed minor evidence of being impacted, but, the results were similar in both hard and soft water.
3. Dissolved silica was the only nutrient that responded differently in hard and soft water, was the only nutrient that was significantly influenced by PCP, and may be a sensitive parameter for detecting low level effects of toxicants in certain systems.
4. Differences between hard- and soft-water treatments were detected in a system function, net oxygen production, when the systems were exposed to 4 mg l^{-1} PCP.
5. Complex systems tests or system parameters may not be as sensitive as single species tests, but this is probably species and toxicant specific.
6. More work needs to be done with other physical-chemical parameters in complex systems exposed to other organic pollutants.

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